REMARKS

Status of the Claims

Claim 10 has been amended to clarify to whom or what subject the recombinant microorganism is administered to.

Claim 19 has been amended to replace "bacterial strain" with --bacterium--.

Claims 20 and 22 have been amended to be properly further limiting to their base claims.

Claim 21 has been amended to indicate the proper antecedence.

Claim 28 has been amended to replace "a nucleotide sequence represented by any of SEQ ID NOS. 1, 2 or 3" with --a nucleotide sequence of SEQ ID NO. 1, 2 or 3--.

Pursuant to 37 C.F.R. §1.118(a), Applicants respectfully submit that the above amendments do not introduce any new material into the application.

With the present amendments, 12 claims are pending in the application, namely, claims 10, 11, 19-24 and 26-29.

Rejection under 35 U.S.C. § 112

Claims 10, 11, 19-24 and 26-29 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. In response, Applicants have amended claims 10, 19-22 and 28 as helpfully suggested by the Examiner. Consequently, this rejection is overcome.

Rejection under 35 U.S.C. § 103

Claims 10, 11, 19-24, 27 and 29 stand rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Podolsky (US 6,221,840) in view of Le Page *et al.* (US 6,221,648) or

Steidler *et al.* (US 6,605,286), Wells *et al.* (Mol. Microbiol. 8:1155-1162, June 1993) and Tran *et al.* (Gut 44:636-642, May 1999). Applicants respectfully traverse this rejection.

Podolsky (US 6,221,840, hereafter "Podolsky") demonstrates that mice lacking endogenous TFF3 are susceptible to dextran sulfate sodium (DSS)-induced colonic injury. Based on this observation, Podolsky contemplates that TFF peptides might be useful in the treatment of gastrointestinal tract disorders and that, because TFF peptides are not degraded within the digestive tract, it is expected that the route of administration will be oral (emphasis added, col. 5, lines 48-49 and col. 15, lines 42-43). Hence, Podolsky only speculates but does not conclusively demonstrate that orally administered TFF peptides can actually treat intestinal disorders.

The teaching of Podolsky might have motivated one of ordinary skill in the art to try to treat gastrointestinal tract disorders using orally administered TFF peptides. However, apart from the observation that TFF peptides are not degraded within the digestive tract, there is no indication in Podolsky that such treatment would have worked. The presumed stability of orally administered TFF peptides in the gastrointestinal tract is only one criterion which may influence their therapeutic use. Therefore, as such Podolsky cannot provide a reasonable expectation of success for oral administration of TFF peptides in treating gastrointestinal tract disorders in the absence of experimental evidence.

Le Page *et al.* (US 6,221,648, hereinafter "Le Page") suggests that *Lactococci* can be used to produce heterologous polypeptides by means of a T7 or T7-like RNA polymerase gene under the control of an inducible promoter. As further described therein, Le Page suggests that the biologically active polypeptides can be delivered in encapsulated form as oral or topical medicaments, or as vaccines. There is no mention or suggestion of using trefoil peptides

delivered *via Lactococcus* for treating gastrointestinal inflammatory diseases of the gut or the colon. In fact, there is no suggestion that using the *Lactobacteria* described by Le Page to recombinantly deliver trefoil peptides *in situ* to the gut will result in a successful treatment of gastrointestinal inflammatory diseases.

Steidler *et al.* (US 6,605,286, hereafter "Steidler") describes that bacteria, such as *Lactococcus*, can be used to deliver/administer bioactive proteins *in situ*. While Steidler does teach a delivery method for pharmacologically active proteins, there are many delivery methods known in the art. Steidler does not suggest that its method would be particularly suited for delivering trefoil peptides to the intestines.

Wells *et al.* (Mol. Microbiol. 8:1155-1162, 1993, hereafter "Wells") demonstrates that *L. lactis* can produce TTFC (tetanus toxin fragment C), and that such pre-loaded bacteria can be used in conjunction with vaccine antigen delivery. The results only show that *L. lactis* is capable of expressing substantial quantities of heterologous protein antigen, and that *L. lactis* is capable of presenting the expressed antigen to the immune system of a mouse in an immunogenic form. There is no mention or suggestion of recombinantly delivering trefoil peptides *via* bacteria in order to successfully treat diseases of the gastrointestinal tract, especially the colon.

Tran et al. (Gut 44:636-642, May 1999, hereafter "Tran") demonstrates successful treatment of a rat model of colitis with intra-rectally administered TFF2. However, intra-rectal administration delivers the TFF peptides at the site of action and avoids the journey through gastrointestinal tract that orally administered TFF must undergo. That is, Tran teaches a different delivery method from the present invention. The disclosure of Tran does not suggest

oral administration of TFF peptides, let alone, suggest that oral administration would have a reasonable expectation of success.

Additionally, none of the other previously cited prior art references conclusively demonstrate treatment of intestinal disorders using orally administered TFF peptides. For example, Jorgensen *et al.* (Regul Pept. 1982, 3:231-43, hereafter "Jorgensen") suggests that PSP (TFF2) can be used to treat gastro-duodenal ulcers, but does not demonstrate that this would actually work. Chinery *et al.* (Clinical Science 88: 401-403, 1995, hereafter "Chinery") shows protection against indomethacine-induced gastric damage in rats by systemically applied combination of TFF3 and epidermal growth factor (EGF). Chinery further describes that TFF3 demonstrates no such effect when administered alone. Playford et al. (PNAS 93:2137-2142, 1996, hereafter "Playford") demonstrates that transgenic mice overexpressing human pS2 (TFF1) are resistant to intestinal damage. However, Playford does not extend their findings to orally administering TFF peptides to treat intestinal disorders. It is clearly obvious that over-expression of TFF by the transgenic mice itself differs from oral administration of exogenous peptides to such animal.

Taken together, the cited prior art references do not provide a single demonstration of a successful treatment of intestinal disorders using orally administered TFF peptides. At most, the prior art including Podolsky might have motivated one of ordinary skill in the art to try to treat gastrointestinal tract disorders using orally administered TFF peptides, but does not provide a reasonable expectation that such treatment would be efficacious.

According to MPEP § 706.02(j), for a claim to be obvious, there must be a) a suggestion or motivation to combine reference teachings, b) a reasonable expectation of success, and c) the references must teach all of the claim limitations, *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438

(Fed. Cir. 1991). Obvious-to-try is not to be equated with obviousness under USC § 103, and is not the standard. *See Gillette Co. v. S.C. Johnson & Son, Inc.*, 919 F.2d 720, 16 USPQ2d 1923 (Fed. Cir. 1990), and *Ecolochem, Inc. v. Southern California Edison Co.*, 227 F.3d 1361, 56 USPQ2d 1065 (Fed. Cir. 2000). For a prior art reference to render the claimed invention obvious, there must have been, at the time the invention was made, a reasonable expectation of success in applying the prior art's teachings. *See Life Technologies, Inc. v. Clontech Laboratories, Inc.*, 224 F.3d 1320, 56 USPQ2d 1186 (Fed. Cir. 2000), and *Micro Chem, Inc. v. Great Plains Chem. Co.*, 103 F.3d 1538, 1547, 41 USPQ2d 1238, 1245 (Fed. Cir. 1997). Reasonable expectation of success is assessed from the perspective of the person of ordinary skill in the art. It is impermissible use of hindsight to use the inventors' success as evidence that the success would have been expected. S*ee Life Technologies, Inc. v. Clontech Laboratories, Inc.*, 224 F.3d 1320, 56 USPQ2d 1186 (Fed. Cir. 2000), and *In re Kotzab*, 217 F.3d 1365, 1369, 55 USPQ2d 1313, 1316 (Fed. Cir. 2000).

In the present case, treating gastrointestinal tract disorders using orally administered TFF peptides might be obvious-to-try based on the prior art including Podolsky; however, the prior art does not provide a reasonable expectation of success. In fact, although the teaching of Poulsen et al. (Gut 45: 516-522, 1999, hereafter "Poulsen (1999)") was not yet available at the time of the filing of the present application, Poulsen (1999) as a supporting document demonstrates that orally administrated trefoil factor 2 (TFF2) is apparently fermented and degraded in the caecum by bacteria and no intact TFF2 can be detected in the colonic contents. Poulsen (1999)'s data suggest that orally administered TFF2 would have no effect in the colon.

Furthermore, the Examiner asserts that a skilled artisan, aware that oral administration of TFF peptides is effective in treating gastrointestinal tract disorders based on the teaching of

Podolsky (which is not true as discussed above), would find it obvious to deliver such peptides using a recombinant bacterium. Applicants respectfully disagree. There were no motivations to do so and no reasonable expectation of success even if there were motivations to do so at the time of the filing of the present application.

First, on the effective date of the present application, one of ordinary skill in the art would have been motivated by Podolsky to orally administer TFF peptides to treat gastrointestinal tract disorders. Due to the fact that oral administration seemed to be the simplest manner and the speculation that it might work, there existed no motivation for one of ordinary skill in the art to try other ways of delivery. Even though there existed a motivation, he or she would prefer systemic delivery which was shown by Poulsen *et al.* (Gut 43:240-247, 1998, hereafter "Poulsen (1998)") to be more efficacious than oral delivery. A copy of Poulsen (1998) is enclosed herewith for the Examiner's convenience. In addition, delivery by recombinant microorganisms entails a number of additional steps, which would represent an undue burden given that there was no incentive to depart from oral administration of the peptides.

Secondly, the state of the art at the time of filing of the present application indicates that microbial delivery of TFF peptides would not have a reasonable expectation of success since it could not guarantee that sufficient amount of the peptides could be delivered to the desired site. In particular, both Podolsky and Tran teach that TFF peptides are expressed specifically and abundantly at the mucosal surface of the gastrointestinal tract and that their expression is even more enhanced at the proximity of the injured bowel. One of ordinary skill in the art would reasonably expect that if they were to have any effect, exogenous TFF peptides would need to be administered in quite large quantities.

The need for large quantities of exogenous TFF peptides for treating intestinal conditions is clearly reflected in Tran, which teaches the intra-rectal administration of high quantities of TFF2, i.e., 2.5 mg/kg/day. This would amount to a daily dose of 175 mg for a human of 70 kg. It would be reasonably expected that oral administration would require even larger quantities compared to intra-rectal administration. Additionally, Tran teaches the administration of TFF peptides after maximal damages have occurred. It would be reasonably expected that larger doses would be needed for the treatment of a still progressing condition.

Furthermore, the need for relatively large quantities of TFF peptides is also clear from Podolsky, which teaches that oral TFF peptides must be administered in a dose of 1 to 500 mg, taken once to three times a day (see col. 15, lines 42-43). One of ordinary skill in the art would understand from Podolsky's teaching that a minimum dose is 1 mg per day and that more serious conditions would likely require increasing amount of TFF peptides, possibly as large as 500 mg three times a day.

To further support the notion that a relatively large quantity of TFF peptides would be needed in order for oral administration to be efficacious, Applicants ask for the Examiner's consideration of Poulsen (1998), which has a publication date of August 1998 and is a valid prior art reference.

In particular, Poulsen (1998) demonstrates that pre-treatment of rats with oral SP or ITF protects against gastric injury (page 240, right column, end of second paragraph), and that subcutaneous injections of spasmolytic peptide (SP) in very low doses to rats protects against indomethacine induced gastric damage, whereas similar doses of oral SP were without any effect (page 240, right column, 4th paragraph). Poulsen (1998) further demonstrates that subcutaneously administered SP is able to protect rats against gastric ulcerations in a dose of less

than 1% of the dosage found to be effective following oral administration (page 245, right column, 4th paragraph).

Poulsen (1998)'s demonstration suggests that much lower amounts of SP are needed for subcutaneous injection than for oral administration, because in the former case the peptide is distributed via the circulatory system, whereby direct binding of the peptide to intracellular mucus due to the high affinity of the peptide to the mucin glycoproteins is avoided (page 245, right column, 5th paragraph). Poulsen (1998) further suggests that when SP is given orally, the luminal side of the mucus layer, i.e., the part going to be desquamated next, is exposed to the cross linking effect of the SP and possibly only a minor proportion of the peptides penetrates into the deeper layers (page 246, right column, last paragraph). Hence, there is a clear suggestion in Poulsen (1998) on the effective filing date of the present application that oral administration of TFF peptides might be problematic and might not effectively deliver the TFF peptides to the site of action, while circulating SP is targeted to be delivered to its binding site and possible sites of action in the gastrointestinal tract more effectively (page 247, last paragraph).

In view of the above remarks, one of ordinary skill in the art would expect that considerably large quantities of TFF peptides would have been needed to treat gastrointestinal tract disorders when administered orally. However, the teaching of Steidler *et al.* (Infect Immun. Jul 66(7): 3183-3189, 1998, hereafter "Steidler (1998)") suggest that such considerably large quantities of TFF peptides could not be obtained using microbial delivery. In particular, Steidler (1998), a copy of which is enclosed for the Examiner's consideration, predicts in Figure 2 that 5×10^8/ml of *Lactococcus lactis* will produce approximately 0.9 microgram of IL-2 or IL-6. A saturated bacterial suspension, typically containing 2×10^9 bacteria/ml, can thus produce approximately 3.6 μg (4 × 0.9 μg) of IL-2 or IL-6.

Based on the teaching of Steidler (1998), one of ordinary skill in the art would expect that to achieve the therapeutic TFF amount administered by Tran (175 mg for a human subject of 70 kg per day), the human subject should receive 48.6 liter (175,000 μg / 3.6 μg/ml = 48,611 ml) per day of bacterial suspension maintained at ideal conditions for growth. Given that a molecule of IL-2 or IL-6 is twice as heavy than a molecule of TFF and that the bacteria will produce whatever weight molecule at the same rate, a skilled artisan would calculate that a 70 kg of human subject should receive 97.2 liter (2 × 48.6 liter) bacterial suspension per day in order to obtain 175 mg TFF. In addition, given that the conditions for bacterial growth in the gut are obviously much less optimal than in the fine-tuned laboratory conditions, these volumes would likely have to be increased considerably.

Moreover, Tran discloses that the intrarectal administration dose was based on previous *in vivo* studies of damage repair in the stomach, which concerns oral administration. (*See* page 637, right column, first paragraph.) In other words, Tran uses relatively large quantities of trefoils for direct rectal administration. Tran is completely silent about using bacteria as a delivery tool. Even with the disclosures of Steidler, Wells and Le Page already available (as discussed above) as well as Robinson *et al.* (Nature Biotechnol. 15: 653-657, 1997), Pouwels *et al.* (J. Biotechnol. 44: 183-192, 1992) and Pouwels *et al.* (Int. J. Food Microbiol. 41: 155-167, 1998), Tran still did not suggest the use of bacteria as a delivery tool.

Applicants believe that one of ordinary skill in the art contemplating to use microbial delivery of TFF peptides would certainly have cared to make some calculations as to just how much of the bacterial culture is needed for oral administration in order to have an effective amount of the trefoil peptides produced at the site, for example, calculations as described above. He or she would have found that this manner of delivery could never attain the quantities that are

considered to be necessary to achieve the therapeutic effect of TFF peptides based on the state of art. Consequently, he or she would not have been motivated to pursue this route, as no reasonable success was obviously forthcoming.

It is Applicants' assertion that the present finding of TFF-expressing bacteria being therapeutically effective despite the small amounts of TFF peptides that such bacteria can possibly deliver is highly surprising. In fact, on page 17, paragraph [0073], the substitute specification submitted February 26, 2004 states:

> "As can be observed from this figure, the expression of the recombinant gene is quite low. This renders the observed in vivo result surprising since others use purified trefoil peptides in therapies for the repair of gastric and intestinal injury at dramatically higher levels; e.g. Tran et al. (1999) used daily intrarectal application of human recombinant TTF2 at levels of 2.5 mg/kg body weight for five days to obtain a reduction in the inflammatory index of experimentally installed colitis in rats (intracolonic administration of dinitrobenzene sulphonic acid in alcohol)."

Rule 132 Declaration of Lothar Steidler Provides Demonstration of Unexpected Results from Bacteria Delivered Recombinant Trefoil Peptides Compared to Purified Trefoil Peptides Administered Orally

To further support the assertion that the superior efficacy of bacterial TFF delivery in treating gastrointestinal disorders is unexpected and highly surprising, Applicants submit herewith further experimental data that is being made of record in the accompanying Rule 132 Declaration signed by Dr. Lothar Steidler, who is a co-inventor of the instant application.

The comparative data provided in the accompanying Rule 132 Declaration clearly establish that the differences in results between bacterial TFF delivery and direct oral

administration of purified TFF are in fact unexpected and unobvious and of both statistical and practical significance. These unexpected results should be enough to rebut a *prime facie* case of obviousness. *See* MPEP 716.02 to 716.02(g).

In view of the above remarks and Rule 132 Declaration, Applicants respectfully submit that Podolsky in view of Le Page or Steidler, Wells and Tran does not render obvious the instant invention as claimed and accordingly, request that the rejection of claims 10, 11, 19-24, 27 and 29 under 35 U.S.C. § 103(a) be traversed.

Claim 26 stands rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Podolsky (US 6,221,840) in view of Le Page *et al.* (US 6,221,648) or Steidler *et al.* (US 6,605,286), or Wells *et al.* (Mol. Microbiol. 8:1155-1162, June 1993) and Tran *et al.* (Gut 44:636-642, May 1999) as applied to claim 10, and further in view of Silk (WO 82/03329). Applicants respectfully traverse this rejection.

As discussed above, Podolsky, Le Page, Steidler, Wells and Tran, alone or combined, do not teach or suggest treatment of gastrointestinal tract disorders by oral administration of a recombinant microorganism expressing a trefoil peptide *in vivo*. One of ordinary skill in the art would not expect that using a gastric catheter, as per Silk, would overcome the recognized barriers to using trefoil peptides for treating intestinal disorders, as described above. Applicants therefore respectfully request that this rejection be traversed.

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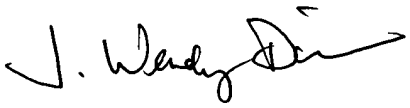
Applicants believe that the present amendments to claims 10, 19-22 and 28 place the case in condition for allowance. The Examiner is encouraged to call the undersigned should any further action be required for allowance.

This document is filed along with a petition for a one-month extension of time. The Commissioner is authorized to deduct the extension fee ($120) from Howrey LLP Deposit Account No. 01-2508/13475.0002.PCUS00. Should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, the Commissioner is authorized to deduct said fees from the same Deposit Account.

Respectfully submitted,



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